



Association of a dominantly inherited hyperphosphorylated paraprotein target with sporadic and familial multiple myeloma and monoclonal gammopathy of undetermined significance: a case-control study

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Summary

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Background Chronic antigenic stimulation might have a role in the pathogenesis of monoclonal gammopathy of unknown significance (MGUS) and multiple myeloma. The aim of this study was to search for factors underlying the autoimmunogenicity of paratarg-7, a frequent antigenic target of paraproteins in MGUS and multiple myeloma.

Methods Between January, 2005, and February, 2009, serum and peripheral blood cells were obtained from consecutive patients with MGUS or multiple myeloma and healthy blood donors, and paratarg-7 was analysed by DNA sequencing, SDS-PAGE, isoelectric focusing, and western blotting.

Findings Mutations or polymorphisms of paratarg-7 were not noted, but hyperphosphorylation was detected in 35 (13·9%) of 252 patients with MGUS or multiple myeloma, all of whom had an anti-paratarg-7-specific paraprotein. Analysis of eight families showed that hyperphosphorylated paratarg-7 is inherited in a dominant fashion, and that carriers of hyperphosphorylated paratarg-7 have an increased risk of developing MGUS and multiple myeloma (odds ratio [OR] 7·9, 95% CI 2·8–22·6; $p=0\cdot0001$).

Interpretation Familial MGUS and multiple myeloma were associated with a dominant inheritance of hyperphosphorylated paratarg-7, enabling family members at increased risk for MGUS or multiple myeloma to be identified. That only patients with MGUS or multiple myeloma who are carriers of hyperphosphorylated paratarg-7 had a paratarg-7-specific paraprotein suggests that the hyperphosphorylation of paratarg-7 induces auto-immunity and is involved in the pathogenesis of MGUS and multiple myeloma; for example, by chronic antigenic stimulation.

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Introduction

Multiple myeloma is a B-lymphocyte-derived malignancy characterised by a monoclonal proliferation of plasma cells that produce a clonal immunoglobulin. Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic precursor condition that often precedes multiple myeloma.¹ Recent studies show that family members of patients with MGUS or multiple myeloma have a two-to-three-fold higher risk of developing MGUS and multiple myeloma.^{2,3} Environmental influences, chance occurrence, and inherited factors might all contribute to familial clusters. The incidence of multiple myeloma and the prevalence of MGUS are also reportedly two-to-three-fold higher among black individuals than in white individuals.^{4–6} However, until now, the pathogenesis of MGUS and multiple myeloma has remained unclear.

A causal relationship between MGUS and multiple myeloma and chronic antigenic stimulation has been suggested by the results of several studies,^{7–11} so identifying the antigenic stimuli of B-cell neoplasms might be important. The antigenic targets of the paraproteins identified to date were discovered accidentally as a result of clinical symptoms caused by

the paraprotein (eg, chronic cold agglutinin disease or cryoglobulinaemia,¹² or bleeding disorders¹³), because of interference of the paraprotein with laboratory tests ordered for the clinical work-up of the patient (eg, HIV-1 p24 antigen in an HIV-infected patient with myeloma¹⁴), and by screening paraproteins against pre-defined antigens (eg, anti-streptolysin, anti-DNA, and anti-IgG¹⁵).

The first systematic studies covering a broad spectrum of potential antigens used SEREX (serological identification of antigens by expression cloning), which allows putative antibody–antigen interactions to be systematically screened, even if neither the antigen nor the antibody are known.¹⁵ cDNA libraries derived from human testis, lung cancer, and breast cancer, bovine and porcine muscle, and wheat germ were expressed in *Escherichia coli* and investigated by SEREX for reactivity with paraproteins from the sera of 114 patients with MGUS or multiple myeloma. More than 6×10^8 paraprotein–antigen interactions were probed, resulting in the identification of only four antigens, each recognised by the paraprotein of only one patient.^{16,17} In a complementary approach, we screened a human fetal brain-derived macroarray with the IgA or IgG

paraprotein-containing sera of 192 consecutive patients with MGUS or multiple myeloma.^{18–29} (15·1%) of 192 paraproteins from patients with MGUS or multiple myeloma reacted with paratarg-7, a protein of unknown function, which is expressed in all human tissues.¹⁹ Paratarg-7 is identical to STOML2 (stomatin [EPB72]-like), also known as HSPC108 or stomatin-like-protein and SLP-2, which has also been reported to be expressed in all human tissues, and overexpressed^{20,21} in several cancers.²² Other investigators have reported that paratarg-7 modulates T-cell activation,²³ and a recent report claimed that SLP-2 is required for stress-induced mitochondrial hyperfusion.²⁴ In this study, we set out to characterise paratarg-7 in order to explain its frequent auto-immunogenicity in patients with MGUS and multiple myeloma.

Methods

Patients

This study was approved by the local ethical review board (Ethikkommission der Ärztekammer des Saarlandes) and was done according to the Declaration of Helsinki. Between January, 2005, and February, 2009, consecutive patients treated at Saarland University Medical School in whom serum protein electrophoresis had identified a monoclonal spike that was shown to contain a monoclonal IgA or IgG paraprotein by immunofixation were recruited to the study. After obtaining written consent, blood was also drawn from 100 healthy employees on their yearly check-up by the University of Saarland Medical School medical officer, and 100 anonymous healthy blood donors of Saarland University Medical School Institute of Transfusion Medicine. Healthy was defined as having no monoclonal immunoglobulin by serum electrophoresis and immunofixation, and being healthy as diagnosed by the Medical Officer or in the pre-donation check-up. Controls were not matched for sex or age. Whenever possible, human materials were obtained during routine diagnostic or therapeutic procedures and stored at –80°C until use. Written informed consent was obtained from patients, their relatives, and controls for studying paratarg-7 in lysates of their whole peripheral blood.

All relatives identified by pedigree analysis were contacted through the patients. Human materials were obtained from 35 relatives from eight families of patients with MGUS or multiple myeloma (seven families with multiple myeloma and one family with two sisters that had MGUS).

	MGUS	Multiple myeloma	Total
IgA	2/24	4/21	6/45
IgG	15/117	14/90	29/207
Total	17/141	18/111	35/252

Table: Detection of paratarg-7 specific paraproteins in 252 consecutive patients with MGUS and multiple myeloma

Procedures

For western blot analyses, blood samples were centrifuged and washed with phosphate buffer solution (PBS). The pellet was resuspended in 8 mol/L urea, 0·1 mol/L phosphate, 10 mmol/L TrisHCl pH 8, and 0·1% NP40 and incubated at 20°C for 15 min.

For isoelectric focusing analyses (IEF), blood samples were centrifuged (three times for 1 min at 4500×g) and washed with PBS followed by lysis in buffer containing 8 mol/L urea, 10 mmol/L NaH₂PO₄, 0·01 mol/L TrisHCl, and 0·1% NP40 (15 min, 20°C) and stored at –20°C until use. Equal volumes of sample and loading buffer were mixed. Samples were analysed by IEF on a gel with a fixed pH gradient (pH 3–10) according to the manufacturer's instructions (Novex pH 3–10, Invitrogen, Germany, Karlsruhe) followed by an immunoblot screening.

For immunoblotting analyses, after lysates from whole peripheral blood were separated by IEF or SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis), the proteins were transferred to an Immobilon-P polyvinylidene difluoride membrane (PVDF; Millipore Immobilon, Eschborn, Germany) by semi-dry blotting. The membrane was blocked overnight at 4°C in TBST/milk buffer (10% [v/v] milk in 10 mmol/L TrisHCl, pH 7·5, 150 mmol/L NaCl, and 0·1% [v/v] Tween 20), washed and incubated for 1 h at room temperature with serum in TBST (paraprotein-containing serum from patients at a dilution of 1:10⁸ and from controls at a dilution of 1:10³). After three washings in TBST, the membranes were incubated for 1 h at room temperature with mouse anti-human IgG–POX antibody (BioRad, California, USA) diluted 1:3000 in TBST. The membranes were then washed in TBST, followed by detection using Pharmacia's ECL (enhanced chemiluminescence) system (General Electrics, Connecticut, USA).

For phosphatase treatment of paratarg-7, blood samples were centrifuged and washed with PBS followed by lysis in LS buffer (10 mmol/L TrisHCl pH 8, 30 min, 4°C). After increasing the concentration of TrisHCl to 100 mmol/L, alkaline phosphatase was added (1 U/μL per 500 μL lysate) and incubated at 37°C overnight. The phosphatase was inactivated by heating at 80°C for 10 min. Equal volumes of sample and loading buffer were mixed, followed by IEF and immunodetection as described above.

The paratarg-7 ELISA using full-length recombinant paratarg-7 was done as described previously.¹⁸

Role of the funding source

The sponsor had no influence on the design and conduct of the study, or on the writing of the manuscript. The corresponding author had full access to all of the data and had the final responsibility to submit for publication.

Results

Between January, 2005, and February, 2009, serum samples from 252 consecutive patients with MGUS or

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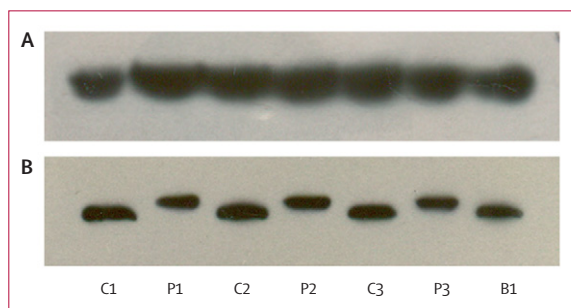


Figure 1: Western blot analysis of paratarg-7 derived from erythrocyte lysates of patients with paratarg-7-reactive paraproteins and healthy controls

Bands of erythrocyte lysates from three patients with anti-paratarg-7 paraproteins (P1–P3), three healthy controls (C1–C3) and one patient with a non-paratarg-7-specific paraprotein (B1) are identical after gel electrophoresis (A) followed by blotting onto nitrocellulose membrane and staining with an anti-paratarg-7 paraprotein. However, after isoelectric focusing (B) the bands of erythrocyte lysates from patients and controls migrate differently.

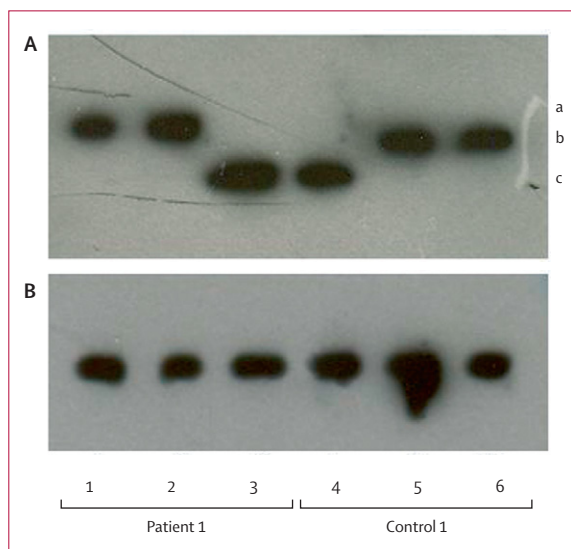


Figure 2: Phosphatase treatment of paratarg-7

Isoelectric focusing (IEF; A): bands of untreated lysates from one patient with anti-paratarg-7-specific paraprotein (patient 1) and from one healthy control (control 1) migrate differently in the IEF (lanes 1 and 6). After incubation with lysis buffer, bands from patient and control also migrate differently (lanes 2 and 5). After treatment of lysates with alkaline phosphatase, bands from patient and control show identical migration patterns. (a) Migration of hyperphosphorylated paratarg-7; (b) migration of paratarg-7 from a healthy donor; (c) migration of dephosphorylated paratarg-7. SDS-PAGE (B): In all cases, no differences in migration are detectable.

multiple myeloma with a monoclonal spike in serum electrophoresis that was confirmed by immunofixation to be a monoclonal IgA or IgG paraprotein were included in this study (table). There were 140 male and 112 female patients. Median age was 66 years (range 35–89 years). There was no difference with respect to age, sex (all patients), progression to multiple myeloma, or stage of the disease, between patients with a paratarg-7 specific paraprotein and those without. Because chromosomal analyses are available from two patients only (patient 13:

IGH-CCND1 rearrangement; and patient 28: 13q14 deletion; see webappendix), it is not yet possible to assess any associations between hyperphosphorylated paratarg-7 and specific chromosomal abnormalities of malignant cells in multiple myeloma. Patients are classed as having MGUS if the first blood sample was obtained while they had still MGUS, even if they later progressed to multiple myeloma. Sera were tested at a dilution of 1:10⁸ for anti-paratarg-7 reactivity using a paratarg-7 ELISA. These series included 192 patients analysed in our previous study.¹⁸ 35 of 252 (13.9%) paraproteins from MGUS or multiple myeloma patients were shown to react with paratarg-7. The anti-paratarg-7 reactivity of these sera had titres ranging from 1:10⁸ to 1:10¹⁰. None of the sera from healthy controls reacted at a dilution of 1:10² or less. Lower serum dilutions were not tested because they cause too much background noise in the paratarg-7 ELISA.

Sequence analysis of paratarg-7 derived from patients who had a paraprotein with specificity for paratarg-7 revealed no differences from paratarg-7 derived from patients with a paraprotein specific to other paratargs or unknown antigenic targets, or from healthy controls (data not shown). These observations exclude the possibility that mutations or polymorphisms are responsible for the autoimmunogenicity of paratarg-7. After gel electrophoresis, whole peripheral blood lysates from patients and healthy controls were subjected to western blot using a paraprotein with anti-paratarg-7 activity as the antibody source. All lysates from patients with a paraprotein with anti-paratarg-7 or non-paratarg-7 specificity and healthy controls showed identical bands in the western blots (figure 1). In addition, lysates from patients and healthy controls were subjected to an IEF separation followed by immunodetection with anti-paratarg-7 paraprotein. Compared with lysates from healthy controls, lysates from all 35 patients with a paratarg-7 specific paraprotein migrated differently in the IEF than lysates from other patients or controls (figure 1). Because the DNA sequences and SDS-PAGE analyses from patients and healthy controls were identical, the differences in behaviour of paratarg-7 seen in the IEF gels suggested that differential post-translational modifications resulted in a different isoelectric point for paratarg-7 derived from patients with a paratarg-7-specific paraprotein. To test whether this difference was due to differential phosphorylation, lysates were treated with alkaline phosphatase, followed by IEF and immunodetection (figure 2). After phosphatase treatment, all lysates showed identical banding, regardless of whether they were derived from healthy controls, from patients having a paraprotein with specificity for paratarg-7, or from patients with a paraprotein that did not bind to paratarg-7. Differential banding in the IEF of healthy controls before and after treatment with alkaline phosphatase showed that paratarg-7 is also phosphorylated in healthy individuals, though to a lesser degree than in patients with MGUS or multiple myeloma

with an anti-paratarg-7 reactive paraprotein. All 35 patients with MGUS or multiple myeloma with an anti-paratarg-7 paraprotein expressed hyperphosphorylated paratarg-7, but hyperphosphorylation was not seen in the 217 patients with multiple myeloma or MGUS whose paraprotein did not bind to paratarg-7 (data not shown).

The members of eight of the 35 families with patients that had paratarg-7-specific paraproteins in their serum gave written consent to be studied for hyperphosphorylated paratarg-7 carrier state. Pedigrees of eight families of patients with MGUS or multiple myeloma with an anti-paratarg-7-specific paraprotein reactive with hyperphosphorylated paratarg-7 were analysed. The pedigree in figure 3 shows the family of a 60-year-old woman with MGUS (I.1). Her younger sister (I.2) also had MGUS. Both sisters had a paratarg-7-specific IgG paraprotein (titre 1:10⁹) and were carriers of hyperphosphorylated paratarg-7. Their brother (I.3) did not have a paraprotein, but isoelectric focusing showed that he was also a carrier of the hyperphosphorylated paratarg-7 protein. In the next generation, both children of our patient (II.1 and II.2) and one daughter (II.6) of family member I.2 carried the hyperphosphorylated protein, but did not have a paraprotein or an anti-paratarg-7 reactivity in their serum at a dilution of 1:10². In the third generation, one of three children (III.4) was a carrier of the hyperphosphorylated paratarg-7 state, but none had a paraprotein in the serum. The pedigrees of another family of one patient with an IgA paratarg-7-specific paraprotein is shown in figure 4, and the pedigrees of six additional families are shown in supplementary figures. In all families, the analysis of relatives of patients with MGUS or multiple myeloma with an anti-paratarg-7-specific paraprotein showed that the hyperphosphorylated state of paratarg-7 is inherited in a dominant fashion. Notably, there are healthy carriers of hyperphosphorylated paratarg-7 who were older than the respective index patient, indicating that factors others than age determine if, and when, a carrier of hyperphosphorylated paratarg-7 develops a paratarg-7-specific paraprotein.

We also studied the prevalence of hyperphosphorylated paratarg-7 by isoelectric focusing and western blot in 200 (unmatched) healthy controls. Compared with the 252 patients with MGUS or multiple myeloma, of whom 35 (13.9%) carried the hyperphosphorylated state of paratarg-7, only four (2%) of 200 healthy blood donors had the same condition. The repetition of serum electrophoresis, immunofixation as well as the light chain ratio excluded the presence of monoclonal immunoglobulins in the serum of these four individuals. This suggests that hyperphosphorylation of paratarg-7 is associated with a significantly increased risk of developing MGUS or multiple myeloma (odds ratio [OR] 7.9, 95% CI 2.8–22.6; $p=0.0001$). Because the median age of the controls was 26 years less than that of the patients with

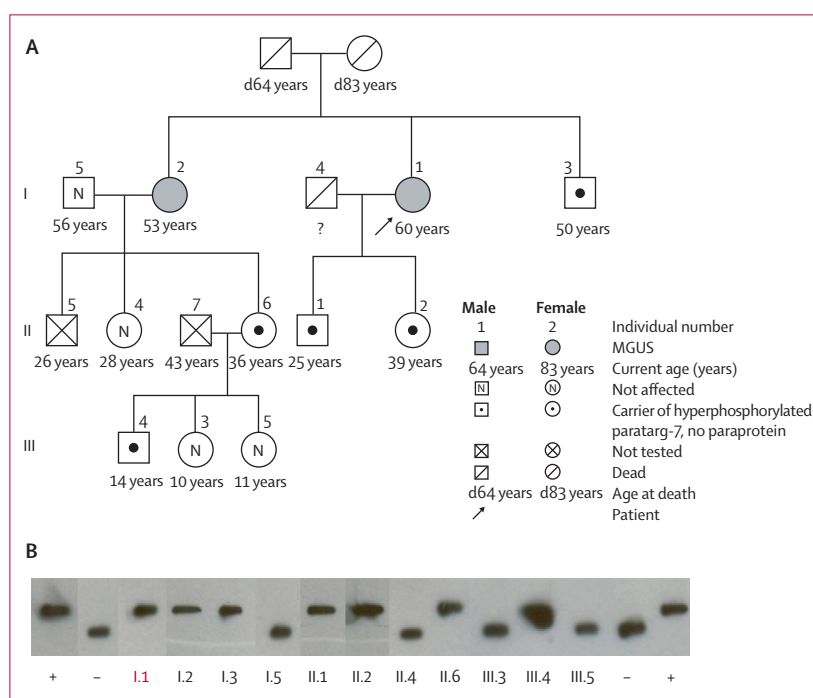


Figure 3: Pedigree of family members of two sisters with MGUS with an IgG paratarg-7-specific paraprotein carrying the hyperphosphorylated paratarg-7

(A) The pedigree shows the family of two sisters with MGUS (I.1 and I.2), both having paratarg-7 reactive paraproteins and carrying the hyperphosphorylated state of this protein. (B) Immunostaining of lysate bands derived from whole peripheral blood lysates from family members carrying wild-type (I.5; II.4; III.3; III.5) and hyperphosphorylated paratarg-7 (I.1; I.2; I.3; II.1; II.2; II.6; III.4) after IEF. The numbers indicate family members in different generations.

MGUS or multiple myeloma (40 vs 66 years), later development of MGUS or multiple myeloma cannot be excluded in the control group.

Discussion

To the best of our knowledge, this is the first study to identify the post-translational modification (hyperphosphorylation) of a molecularly defined structure as the auto-antigenic target of a significant proportion of paraproteins in patients with MGUS or multiple myeloma. Moreover, our results show that the carrier state of hyperphosphorylated paratarg-7 is inherited in a dominant fashion, and is associated with a significantly increased risk for the development of MGUS or multiple myeloma. Hyperphosphorylated paratarg-7 is the first dominantly inherited risk factor identified in a haematological neoplasm, and enables the identification of family members of patients with MGUS or multiple myeloma who have an increased risk of developing MGUS or multiple myeloma.

A causal relationship between MGUS or multiple myeloma and chronic antigenic stimulation has been suggested by the results of several studies.^{7–11} Some reports have shown increased risks for specific immunostimulating conditions (eg, autoimmune diseases, infections, and allergies); however, results have generally

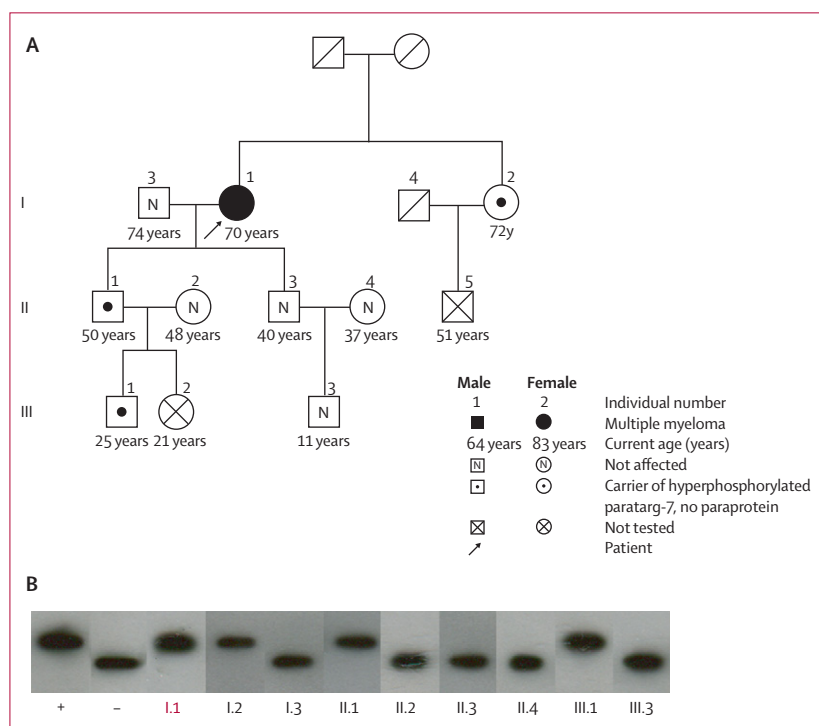


Figure 4: Pedigree of a patient with multiple myeloma with a paratarg-7-specific IgA paraprotein carrying the hyperphosphorylated paratarg-7.

(A) The pedigree shows the family of two sisters (I.1 and I.2), only one of whom has multiple myeloma (I.1) and a paratarg-7-reactive paraprotein, but both carry the hyperphosphorylated state of this protein. The pattern of a hyperphosphorylated paratarg-7 carrier state in this family is also consistent with an autosomal dominant trait. (B) Immunostaining of lysate bands derived from whole peripheral blood lysates from family members carrying wild-type (I.3; II.2; II.3; II.4; III.3) and hyperphosphorylated paratarg-7 (I.1; I.2; II.1; III.1) after isoelectric focusing. The numbers indicate family members in different generations.

been inconsistent.^{4,5,9,10,25–34} Although several previous studies have identified myeloma paraproteins directed against various infectious agents, including bacteria,¹² cytomegalovirus,³⁵ and the p24 antigen of the HIV-1,¹⁴ these studies failed to identify an antigenic target of paraproteins from patients who had signs of chronic antigenic stimulation at a representative frequency. Similarly, approaches to identify paraprotein targets by epitope reconstruction³⁶ were not convincing, because reactions were seen at concentrations of the paraprotein-containing serum of only 1:1250, raising doubts over whether the observed reactions were indeed paraprotein-mediated. Our first systematic studies using SEREX showed paraprotein-mediated reactivity against the identified antigens based on the high reactivity (<1:10⁸) of the paraprotein and the appropriate controls.^{16,17} However, we identified a broad spectrum of auto-antigens, allo-antigens, and heteroantigens as targets of single human paraproteins.^{16,17} In a follow-up study of paraprotein-containing sera from patients with MGUS or multiple myeloma, we used a human fetal brain-derived protein macroarray¹⁸ and found that 15·1% of all paraproteins analysed reacted with paratarg-7. The specificity of the paratarg-7 binding paraproteins was

extensively discussed in our previous study¹⁸ and has recently been confirmed by the cloning of B-cell receptors of two patients with a paratarg-7-specific paraprotein (data not shown). Why paratarg-7 was missed in our earlier study using the SEREX approach,¹⁷ even though it was represented in the cDNA libraries screened in that study, is not clear. It is most likely due to the fact that the epitope of paratarg-7, which reacts with the respective paraproteins, is located at the very beginning of the molecule, and can be easily missed if the full-length protein is not expressed. Since full-length clones are the exception rather than the rule in the SEREX approach, lack of the full-length expression of paratarg-7 was the most likely reason why paratarg-7 was not detected in the earlier study¹⁷ that used a SEREX screen.

The patients with MGUS or multiple myeloma carrying hyperphosphorylated paratarg-7 and those with paraproteins that did not bind to paratarg-7 in this study showed no significant difference with respect to age, sex, and course of disease (data not shown). The frequency of paratarg-7 as a paraprotein target is much higher than expected by chance, and suggests a direct or indirect role for hyperphosphorylated paratarg-7 in the pathogenesis of MGUS and multiple myeloma. Because of the dominant inheritance of the hyperphosphorylated paratarg-7 carrier state, it is useful to study its prevalence in different ethnic groups who are known to have different incidences of the disease. We were able to study paraproteins from 20 patients from Japan (where there is a low incidence of MGUS and multiple myeloma) of whom one was specific for paratarg-7. Similar studies are planned in Africans and African-Americans, in whom the incidence of MGUS and multiple myeloma is high.

We restricted our analysis to paraproteins of the IgG and IgA type because we had too few cases of IgM paraproteins to allow for any solid conclusions. So far, we have not detected any anti-paratarg-7 reactivity of monoclonal IgM paraproteins from 20 patients with Waldenström's macroglobulinaemia. Similarly, we did not detect carriers of hyperphosphorylated paratarg-7 among 50 patients with follicular lymphoma, 50 patients with diffuse large B-cell lymphoma, and 50 patients with Hodgkin's lymphoma (data not shown).

The paratarg-7 protein derived from patients and healthy controls showed no differences with respect to DNA sequence and pattern in western blots, thereby excluding mutations or polymorphisms as a possible reason for its autoimmunogenicity. However, IEF and phosphatase treatment showed that paratarg-7 was hyperphosphorylated in all patients with this paraprotein specificity, compared with paratarg-7 protein from patients with MGUS or multiple myeloma with paraproteins that do not bind to paratarg-7 and healthy controls. IEF before and after phosphatase treatment show that hyperphosphorylated paratarg-7 and wild-type paratarg-7 are both phosphorylated, albeit to different degrees. Recent results from our laboratory show that the

difference between wild-type and hyperphosphorylated paratarg-7 is due to a phosphorylation at a single site (data not shown). Since this single additional phosphorylation increases the molecular weight of paratarg-7 (37 000 Da) by only 90 Da, it is conceivable that this small difference could not be detected by SDS-PAGE, but only by IEF. Because of the dominant inheritance of paratarg-7 hyperphosphorylation, a polymorphism in one of the plethora of kinases is more likely to be responsible for the hyperphosphorylation differences than the deficiency or decreased activity of a phosphatase, which would be compensated for by the second allele.

Family analyses of relatives of patients with MGUS or multiple myeloma with an anti-paratarg-7-specific paraprotein showed that the hyperphosphorylated state of this protein is inherited as a dominant trait. There have been a number of studies of familial multiple myeloma, which have implicated both environmental and inherited factors.^{2,32,37–39} Several reports suggest that gene mutations or genetic polymorphisms might be associated with the risk of multiple myeloma.^{40–42} However, results have been inconsistent, and significant findings have not been replicated convincingly.⁴³ Hyperphosphorylated paratarg-7 is the first molecularly characterised structure that provides a plausible explanation for the familial clustering of cases of MGUS or multiple myeloma, at least in cases with a paratarg-7-specific paraprotein. This was the case in one of our families analysed in this study (figure 3), where two sisters were carriers of hyperphosphorylated paratarg-7 and both had a paraprotein with specificity for this antigenic target. It is now possible to investigate whether previously reported cases of familial MGUS or multiple myeloma^{2,3,5,32} can also be explained by the carrier state of hyperphosphorylated paratarg-7.

The frequency of the carrier state of hyperphosphorylated paratarg-7 among patients with MGUS or multiple myeloma and in healthy controls shows a 7.9 times increased risk of developing MGUS or multiple myeloma for carriers of the hyperphosphorylated protein. This is, to the best of our knowledge, the highest OR for an MGUS or multiple myeloma risk factor reported to date.^{3–5,44} Since the control group (median age 40 years) was 26 years younger than the patients with paraproteins (66 years), we cannot exclude that the four healthy carriers will develop an anti-paratarg-specific paraprotein with longer follow-up. The same applies to family members with the hyperphosphorylated paratarg-7 carrier state. If this were the case, it would further increase the OR. Testing for a hyperphosphorylated carrier state should therefore help identify members of the respective families who have an increased risk of developing MGUS or multiple myeloma. Finally, the fact that hyperphosphorylated paratarg-7 functions as the antigenic target of the paraproteins of all patients with MGUS or multiple myeloma with hyperphosphorylated paratarg-7 suggests that the hyperphosphorylated protein has a role

in the development of sporadic and familial MGUS and multiple myeloma. The hyperphosphorylation of paratarg-7 seems to be the most obvious reason for its autoimmunogenicity. Indeed, it has been shown that phosphoepitopes have a higher binding affinity to the major histocompatibility complex and induce stronger CD8⁺⁴⁵ and CD4⁺ T-cell responses.⁴⁶ By contrast with paratarg-7, none of the other targets of individual paraproteins described by us^{16–18} were hyperphosphorylated (data not shown), suggesting that the post-translational modification of paratarg-7 in the form of a hyperphosphorylation might contribute to its frequency as the antigenic target of a considerable proportion of paraproteins. Whether hyperphosphorylated paratarg-7 induces the development of MGUS or multiple myeloma by chronic antigenic stimulation, or whether it is only a marker or an epiphenomenon of another dominantly inherited susceptibility to develop MGUS or multiple myeloma, can now be investigated in patients and their as yet unaffected relatives.

Contributors

SG and K-DP designed the experiments and helped write the manuscript. MA, NM, and CP did the family investigations, NF and ER undertook and analysed the experiments, and MP designed the study and helped write the manuscript.

Conflicts of interest

K-DP and MP have applied for a relevant patent. All other authors declared no conflicts of interest.

Acknowledgments

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Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Grass S, Preuss K-D, Ahlgrimm M, et al. Association of a dominantly inherited hyperphosphorylated paraprotein target with sporadic and familial multiple myeloma and monoclonal gammopathy of undetermined significance: a case-control study. *Lancet Oncol* 2009; published online Sept 21, 2009. DOI:10.1016/S1470-2045(09)70234-7.

Supplemental material

Table 1: Patient characteristics of the 35 paratarg-7 positive patients

Pat-ID	Gender	Age	MM / MGUS	Paraprotein
1	female	66	MM	IgA-Kappa
2	female	89	MGUS	IgG-Kappa
3	female	71	MM	IgA-Lambda
4	female	81	MM	IgG-Kappa
5	male	64	MGUS	IgG-Lambda
6	male	53	MGUS	IgG-Kappa
7	female	65	MM	IgG-Kappa
8	male	61	MM	IgG-Kappa
9	male	54	MGUS	IgG-Kappa
10	male	67	MGUS	IgG-Kappa
11	male	70	MM	IgG-Kappa
12	male	76	MM	IgG-Lambda
13*	male	36	MM	IgG-Kappa
14	male	81	MGUS	IgG-Lambda
15	male	66	MGUS	IgG-Lambda
16	female	57	MGUS	IgG-Lambda
17	female	83	MM	IgG-Kappa
18	male	53	MM	IgG-Lambda
19	male	68	MGUS	IgG-Lambda
20	male	73	MM	IgG-Lambda
21	male	75	MGUS	IgA-Kappa
22	male	54	MM	IgG-Kappa
23	male	62	MGUS	IgG-Kappa
24	male	73	MM	IgA-Kappa
25	male	64	MM	IgA-Kappa
26	male	69	MGUS	IgG-Kappa
27	female	69	MGUS	IgG-Kappa
28**	female	72	MM	IgG-Kappa
29	male	76	MGUS	IgG-Lambda
30	female	61	MGUS	IgG-Lambda
31	male	58	MGUS	IgG-Kappa
32	female	61	MM	IgG-Kappa
33	male	54	MM	IgG-Kappa
34	female	69	MGUS	IgA-Kappa
35	female	61	MM	IgG-Kappa

Median age: 66 years

* cytogenetic analysis of bone marrow showed an IgH-CCND1 rearrangement.

** cytogenetic analysis of bone marrow showed a 13q14 deletion.

Figure Legends

Figure 5. Pedigree of a multiple myeloma patient with a paratarg-7 specific IgG paraprotein carrying the hyperphosphorylated paratarg-7. The pedigree shows the family of a multiple myeloma patient (I.1) and his sister (I.3), only one having MM (I.1) and a paratarg-7 reactive paraprotein, but both carrying the hyperphosphorylated state of this protein.

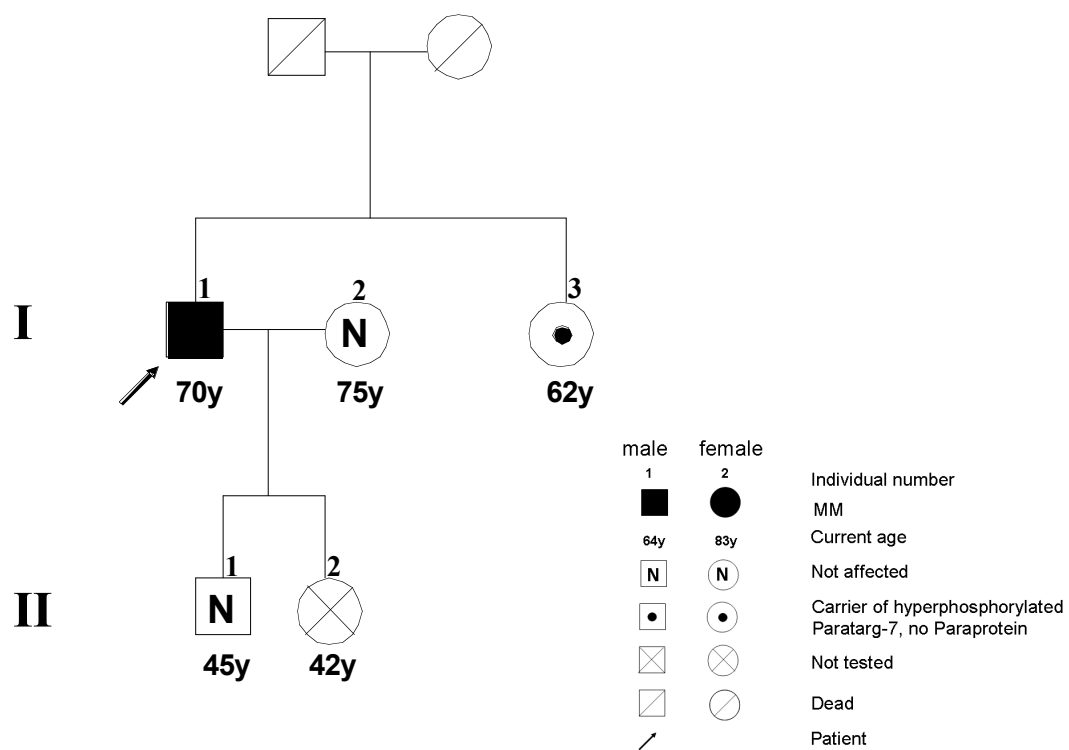
Figure 6. Pedigree of a multiple myeloma patient with a paratarg-7 specific IgA paraprotein carrying the hyperphosphorylated paratarg-7. The pedigree shows the family of a patient (I.1) and his daughter (II.1), only one having MM (I.1) and a paratarg-7 reactive paraprotein, but both carrying the hyperphosphorylated state of this protein.

Figure 7. Pedigree of a multiple myeloma patient with a paratarg-7 specific IgA paraprotein carrying the hyperphosphorylated paratarg-7. The pedigree shows the family of a patient (I.1), her daughter (II.1) and her grandchild (III.1), only one having MM (I.1) and a paratarg-7 reactive paraprotein, but all of them carrying the hyperphosphorylated state of this protein.

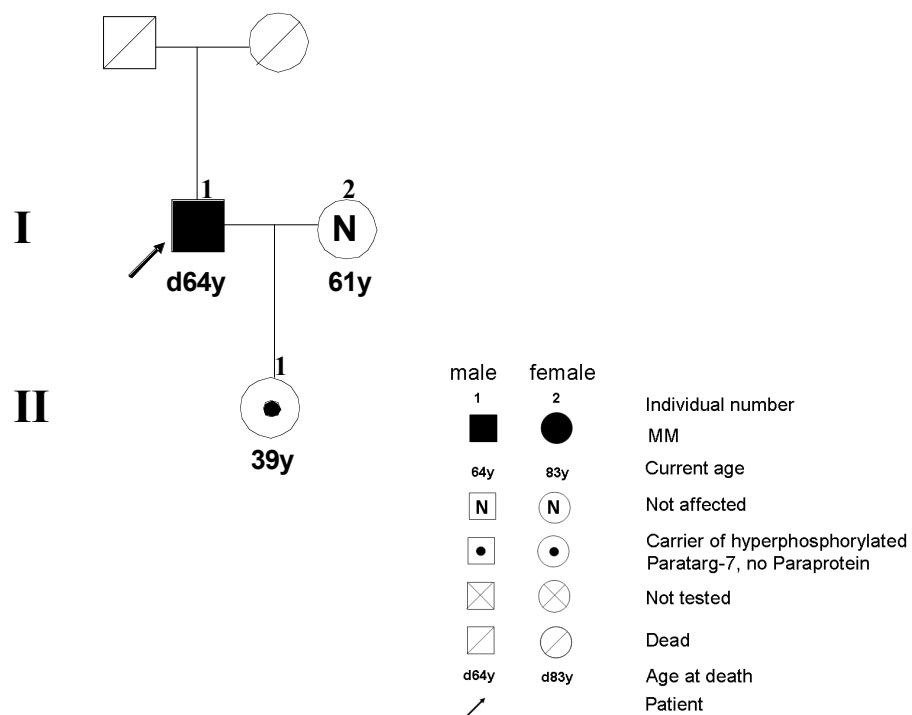
Figure 8. Pedigree of a multiple myeloma patient with a paratarg-7 specific IgA paraprotein carrying the hyperphosphorylated paratarg-7. The pedigree shows the family of a patient (I.1) and her son (II.1), only one having MM (I.1) and a paratarg-7 reactive paraprotein, but both carrying the hyperphosphorylated state of this protein.

Figure 9. Pedigree of a multiple myeloma patient with a paratarg-7 specific IgG paraprotein carrying the hyperphosphorylated paratarg-7. The pedigree shows the family of a multiple myeloma patient (I.1) and his brother (I.2), only one having MM (I.1) and a paratarg-7 reactive paraprotein, but both carrying the hyperphosphorylated state of this protein.

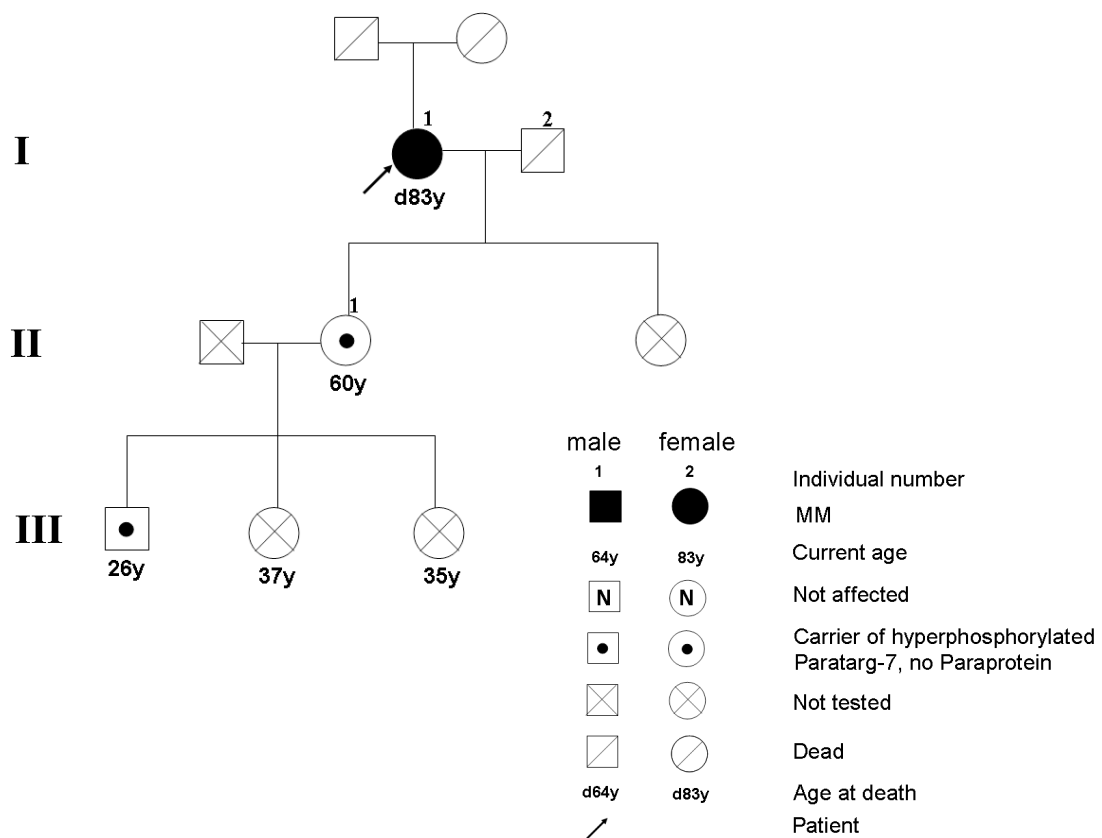
Figure 10. Pedigree of a multiple myeloma patient with a paratarg-7 specific IgG paraprotein carrying the hyperphosphorylated paratarg-7. The pedigree shows the family of a multiple myeloma patient (I.2) and his sister (I.5), only one having MM (I.2) and a paratarg-7 reactive paraprotein, but both carrying the hyperphosphorylated state of this protein. The pattern of a hyperphosphorylated paratarg-7 carrier state is also consistent with an autosomal dominant trait.



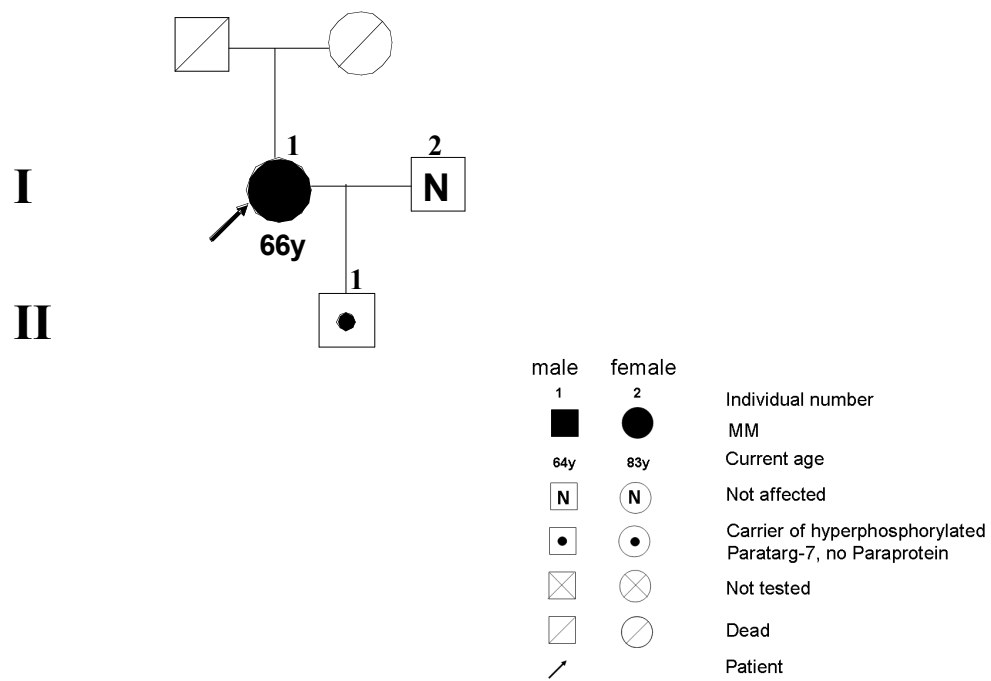
Supplementary Figure 5



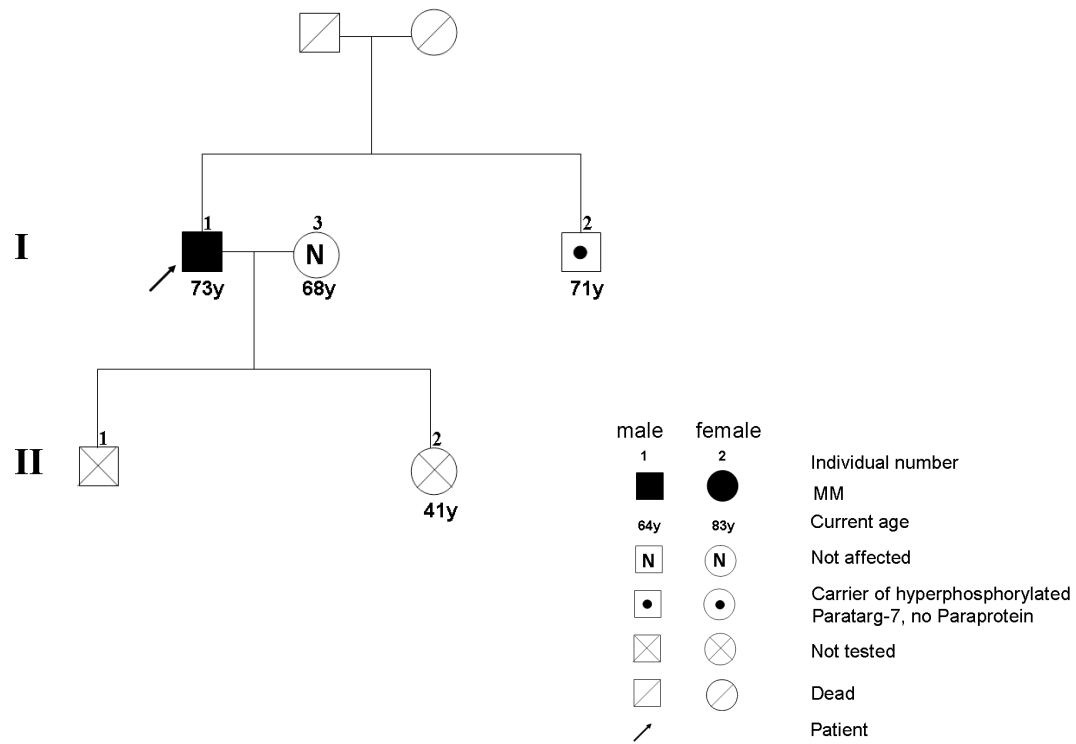
Supplementary Figure 6



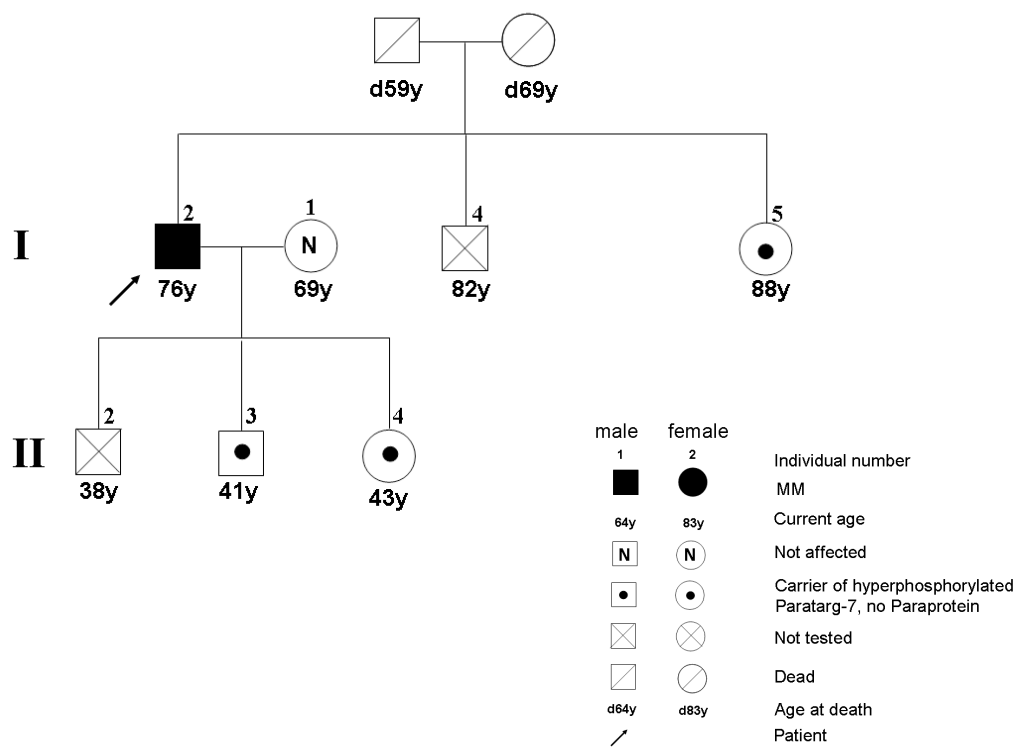
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10